Date of Approval: July 6, 2012

FREEDOM OF INFORMATION SUMMARY

ORIGINAL NEW ANIMAL DRUG APPLICATION

NADA 141-336

AIVLOSIN

Tylvalosin Tartrate
Water Soluble Granules
Swine

Control of porcine proliferative enteropathy (PPE) associated with *Lawsonia intracellularis* infection in groups of swine in buildings experiencing an outbreak of PPE

Sponsored by:

ECO LLC

TABLE OF CONTENTS

I. GENERAL INFORMATION:

A. File Number: NADA 141-336

B. Sponsor: ECO LLC
8209 Hollister Ave.
Las Vegas, NV 89131

Drug Labeler Code: 066916

C. Proprietary Name: AIVLOSIN

D. Established Name: Tylvalosin tartrate

E. Pharmacological Category: Antimicrobial

F. Dosage Form: Water Soluble Granules

G. Amount of Active Ingredient: 62.5 % (w/w) tylvalosin as tylvalosin tartrate

H. How Supplied: 40 g, 160 g, and 400 g sachets, containing 25 g, 100 g, or 250 g tylvalosin respectively, supplied in boxes holding 20, 10, and 5 sachets respectively

I. How Dispensed: By prescription (Rx)

J. Dosage: 50 ppm tylvalosin in drinking water for five consecutive days

K. Route of Administration: Oral via drinking water

L. Species/Class: Swine

M. Indication: Control of porcine proliferative enteropathy (PPE) associated with *Lawsonia intracellularis* infection in groups of swine in buildings experiencing an outbreak of PPE.

II. EFFECTIVENESS:

A. Dosage Characterization:

Two dose determination challenge model studies (Studies EFF.US.040004 and EFF.US.050079) were conducted using a similar protocol to evaluate the effectiveness of 25 ppm, 37.5 ppm, 50 ppm, and 100 ppm tylvalosin (also referred to as acetylisovaleryltylosin during product development) administered in drinking water for five consecutive days for the control of porcine proliferative enteropathy (PPE) associated with *Lawsonia intracellularis*. In each study, a total of 192 female and castrated male pigs, 4 to 5 weeks old, weighing approximately 7 to 14 kg, and sourced from a PPE-free high health farm were randomly allocated on a live weight basis to each of 4 treatment groups. In the first study (EFF.US.040004), the treatment groups included a challenged, non-medicated control group, and challenged groups provided tylvalosin medicated water at targeted inclusion rates of 25 ppm, 50 ppm, and 100 ppm. In the second study (EFF.US.050079), the treatment groups included a challenged, non-medicated control group, and challenged groups provided tylvalosin medicated water at targeted inclusion rates of 25 ppm, 37.5 ppm, and 50 ppm.

Each pig was challenged on Day 0 by intraesophageal or intragastric gavage with an intestinal mucosal homogenate prepared from the affected intestine of a recent North American case of PPE containing approximately $10^{8.5 \pm 0.5}$ infective *L. intracelluaris* organisms. Pigs were examined daily for signs of PPE and treatment was started on Day 6 (designated as DM+1) in both studies, when at least 15% of the total population of pigs at the site were observed to be clinically affected with PPE. A pig was considered to be clinically affected if it had a fecal score of 1 (semi-solid/no blood) or greater. The control pigs received non-medicated water for the duration of the study. All surviving pigs were humanely euthanized and necropsied on DM+22.

Variables measured during the studies included clinical parameters (pig demeanor score, abdominal appearance score, and fecal score), mortality due to PPE, gross PPE lesion score, average daily gain (ADG), and average daily feed intake.

In Study EFF.US.040004, pig demeanor scores, abdominal appearance scores, fecal scores, and ADG of the pigs in all three treated groups (25 ppm, 50 ppm, and 100 ppm tylvalosin) were statistically significantly improved ($P \leq 0.05$) compared to the non-medicated control group. The gross PPE lesion scores of the pigs in the 50 ppm and 100 ppm tylvalosin-treated groups were statistically significantly improved compared to the non-medicated control group; however, the scores of the pigs in the 25 ppm tylvalosin-treated group were not statistically significantly different from the non-medicated control group. Two pigs died or were euthanized due to PPE in the 25 ppm tylvalosin-treated group and no pigs died or were euthanized due to PPE in the other tylvalosin-treated groups. In the non-medicated control group, five pigs died or were euthanized due to PPE.

In study EFF.US.050079, pig demeanor scores, abdominal appearance scores, fecal scores, PPE lesion scores, ADG, and mortality due to PPE in the 37.5 ppm, and 50 ppm tylvalosin-treated groups were statistically significantly improved

($P \leq 0.05$) compared to the non-medicated control group. The abdominal appearance scores, PPE lesion scores, ADG, and mortality due to PPE of the pigs in the 25 ppm tylvalosin-treated group were statistically significantly improved compared to the non-medicated group of pigs; however, the pig demeanor scores and fecal scores of the pigs in the 25 ppm tylvalosin-treated group were not statistically significantly different from the non-medicated controls. In the non-medicated control group, eight pigs died or were euthanized due to PPE. There were no pigs that died or were euthanized due to PPE in the tylvalosin-treated groups in this study.

Based on the results of these studies, a minimum inclusion rate of 50 ppm was selected for the dose confirmation study.

B. Substantial Evidence:

1. Dose Confirmation Model Study

a. Study Number and Title: Study Number EFF.US.050080: "Determination of the efficacy of AIVLOSIN Water Soluble Granules in drinking water for the control of porcine proliferative enteropathy (PPE, ileitis) in pigs experimentally infected with *Lawsonia intracellularis*." August 2006 to November 2006.

b. Investigators and Locations:

(1) Nathan Winkelman, DVM, Swine Services Unlimited, Inc., Rice, MN (Site 1)

(2) Michael Sims, BS, Virginia Diversified Research and Consultants, LLC, Harrisonburg, VA (Site 2)

(3) Heather L Bruce, PhD, Nutreco Agresearch Canada (formerly Maple Leaf Foods Agresearch), Buford, Ontario, Canada (Site 3)

c. Study Design

1) *Objective*: To evaluate the clinical effectiveness of tylvalosin tartrate administered in drinking water for the control of porcine proliferative enteropathy (PPE, ileitis) associated with *L. intracellularis* in pigs.

2) *Test Animals*: A total of 432 healthy weaned commercial crossbred barrows and gilts, approximately 5 weeks old, and weighing approximately 8 to 15 kg, were enrolled on Day -2 (Site 2) or Day -3 (Sites 1 and 3). Pigs were obtained from separate high herd health swine sources for each study site. Animals were determined to be free of *L. intracellularis* based on fecal polymerase chain reaction (PCR) and serological immunoperoxidase monolayer assay (IPMA) testing on 48 randomly selected pigs (2 per pen) at each site.

3) *Experimental Design*: The study was conducted at three independent sites using a randomized complete block design, with the pen as the experimental unit. At each site, 144 pigs were ranked on the basis of body weight and gender and assigned to one of eight gender blocks

(eighteen animals of the same gender and similar weights). The pigs within a gender block were assigned at random to three pens (six animals per pen). One pen from each block was randomly assigned to either the challenged, 50 ppm tylvalosin-treated group; the challenged, 75 ppm tylvalosin-treated group; or the challenged, non-medicated control group.

On Day 0, all pigs were challenged with a mucosal homogenate containing *L. intracellularis* that induces representative disease in challenged pigs. The mucosal homogenate was initially collected in 2005 from a North American finishing pig with a field case of porcine hemorrhagic enteropathy (PHE), an acute, hemorrhagic form of PPE. Pigs were dosed by intragastric gavage with approximately 1.8×10^8 to 1.3×10^9 *L. intracellularis* organisms.

4) *Test Article Administration*: The test article was AIVLOSIN Water Soluble Granules, mixed with water at intended concentrations of 50 ppm and 75 ppm tylvalosin. Treatment was initiated when 15% of the total population of study pigs at the study site was observed to be clinically affected with PPE in a single day at least 3 days after challenge. Treatment was initiated between four and six days post-challenge across all sites. A pig was considered clinically affected when it had a fecal score of ≥ 1 (see score definitions below). Pigs received tylvalosin-medicated water continuously for five consecutive days. The control pigs received non-medicated water for the duration of the study.

5) *Measurements and Observations*: Beginning on Day -2 (Site 2) or Day -3 (Sites 1 and 3), pigs were examined and scored daily for three clinical parameters (abdominal appearance, demeanor, and fecal score) using the following scales.

Abdominal Appearance Score:

0 = normal,
1 = moderately gaunt,
2 = severely gaunt.

Demeanor Score:

0 = normal,
1 = slightly to moderately depressed, listless, will stand,
2 = severely depressed; may or may not be recumbent.

Fecal Score:

0 = no diarrhea,
1 = semi-solid, not formed,
2 = watery stool, <50% water, runs through the floor slats,
3 = profuse projectile diarrhea, >50% water.

The percent abnormal pig days for each clinical score (abdominal appearance, demeanor, and fecal score) was calculated by summing the total number of days with an abnormal score of ≥ 1 for all pigs in a

pen and dividing this numerator by the sum of the days for which each pig was alive. The results were presented as a mean of the pens in each group for the following time periods: DM+1 through DM+7, DM+8 through DM+14, and DM+15 through DM+21.

Average Daily Gain (ADG) per pen: Individual pig live weights were recorded on DM+1, DM+5, and at necropsy on DM+22 or upon death/removal from the study. ADG was calculated on a pen basis as follows:

(Sum of weight gains between DM+1 and DM+22 or upon death or removal)/(total number of pig alive days in the period including number of alive days for removed animals).

Gross PPE Lesion Score: Lesion scores were recorded at necropsy (DM+22 or upon death/removal) for the jejunum, ileum, cecum, and colon of each pig using the following scale.

0 = Normal,
1 = Mild porcine intestinal adenomatosis (PIA), edema, and hyperemia,
2 = Moderate PIA edema/hyperemia/reticulated serosa and mucosa,
3 = Severe PIA edema/hyperemia/reticulated serosa and mucosa/gross thickening of the mucosa/blood or fibrin/necrotic enteritis.

A mean lesion score across the jejunum, ileum, cecum, and colon was calculated for each pig and a mean lesion score per pen was calculated as follows: (sum of pig mean lesion scores)/(number of pigs remaining in data analysis).

Mortality: Pigs were counted as mortalities due to PPE if they died or were euthanized between DM+1 and the day of necropsy (DM+22) and had lesions typical of PPE at necropsy.

Other data recorded during the study: Pen feed consumption data and samples to determine the presence of *L. intracellularis* were also collected during the study.

6) *Criteria to demonstrate effectiveness:* At least one of the following sets of criteria had to be met in order to demonstrate effectiveness:

(a) At least two of the three clinical parameters (pig demeanor score, abdominal appearance score, and fecal score) in the period DM +15 to DM +21 are statistically significantly improved ($P \leq 0.05$) in the tylvalosin-treated group compared to the non-medicated control group – AND – mortality due to PPE is not statistically significantly higher ($P \leq 0.05$) in the tylvalosin-treated group compared to the non-medicated control group.

OR

(b) Gross PPE lesion scores are statistically significantly improved ($P \leq 0.05$) in the tylvalosin-treated group compared to the non-medicated control group – AND – average daily gain (ADG) is numerically improved in the tylvalosin-treated group compared to the non-medicated control group – AND – mortality due to PPE is not statistically significantly higher ($P \leq 0.05$) in the tylvalosin-treated group compared to the non-medicated control group.

7) *Statistical Analysis*: For each of the clinical parameters (pig demeanor score, abdominal appearance score, and fecal score), the arcsine square root of the pen means for percent abnormal pig days (abnormal = score of ≥ 1) was analyzed for the study periods DM+1 through DM+7, DM+8 through DM+14, and DM+15 through DM+21, using a linear mixed model. Fixed effects were treatment, period, and period by treatment interaction. Random effects were site, block within site, site by treatment interaction, and block by treatment interaction within site. Significance of treatment comparisons was assessed within each of the study periods using model based two-sided t tests. The mean of the pig mean lesion scores for each pen and ADG for each pen (kg/pig/day) were analyzed with a linear mixed model with a fixed effect for treatment and random effects of site, block within site, and site by treatment interaction. If the test for overall differences was significant, pairwise comparisons among the treatments were made using model-based two-sided t tests. Mortality was analyzed with Fisher's Exact Test. All testing was done at the 0.05 level of significance.

d. Results: The results for the 50 ppm tylvalosin-treated group are reported below. The results for the 75 ppm tylvalosin-treated group are not reported because of significant water assay deviations from the intended concentration.

1) *Clinical Parameters:* The results from the study period DM +15 to DM +21 were used in the evaluation of effectiveness. At Sites 1 and 2, percent abnormal pig days for demeanor score was statistically significantly improved in the 50 ppm tylvalosin-treated group compared to the control group ($P = 0.0132$ at Site 1 and $P = 0.0177$ at Site 2). Statistically significant differences in percent abnormal pig days for abdominal appearance score ($P < 0.0001$) and fecal score ($P = 0.0017$) were seen between control and 50 ppm tylvalosin-treated groups at Site 2. Differences in percent abnormal pig days for abdominal appearance score and fecal score between control and 50 ppm tylvalosin-treated groups at Site 1 were not statistically significant ($P = 0.2837$ and $P = 0.0968$ for abdominal score and fecal score respectively). The clinical parameter results from Site 3 were not used in the evaluation of effectiveness due to a protocol deviation; therefore, a combined analysis of clinical parameter results across all sites was not performed. The site specific results from Site 1 and Site 2 are summarized in Table II.1.

Table II.1. Percent Abnormal Pig-Days for Clinical Parameters[1]

Site	Treatment Group	Fecal Score (%)	Demeanor Score (%)	Abdominal Appearance Score (%)
1	50 ppm tylvalosin	37.8[a]	0.9[a]	23.2[a]
1	Control	49.9[a]	8.7[b]	33.1[a]
2	50 ppm tylvalosin	7.3[a]	0.1[a]	1.4[a]
2	Control	29.4[b]	5.3[b]	22.3[b]

[1] Within each column at a given site, values with different superscripts indicate statistically significant differences between treatment and control groups ($P \leq 0.05$).

2) *Mortality*: Across all sites, the mortality (0%) in the 50 ppm tylvalosin-treated group was statistically significantly lower ($P < 0.0001$) than the mortality (14.9%) in the non-medicated control group.

3) *Gross PPE lesion scores*: Across all sites, the mean gross PPE lesion score (0.24) in the 50 ppm tylvalosin-treated group was statistically significantly lower ($P = 0.0103$) than the mean gross PPE lesion score (0.69) in the non-medicated control group.

4) *ADG*: Across all sites, the pen ADG (0.48 kg/pig/day) in the 50 ppm tylvalosin-treated group was numerically greater than the ADG (0.31 kg/pig/day) in the non-medicated control group.

e. Adverse Reactions: No adverse reactions attributable to the test article were reported.

f. Conclusions: The results of this study indicate that 50 ppm tylvalosin given continuously for five consecutive days in water is effective for the control of PPE associated with *L. intracellularis*.

III. TARGET ANIMAL SAFETY:

A. Margin of Safety Study:

1. Study Number and Title: Study Number TAS.UK.100219: "Margin of Safety Study of AIVLOSIN (Tylvalosin) Water Soluble Granules in Drinking Water in Pigs." November 2010, to February 2011.

2. Study Director and Location of Study

   Ciara Vance, BSc; Charles River Laboratories, Tranent, Edinburgh, United Kingdom

3. Study Design

   a. *Objective*: To evaluate the margin of safety for AIVLOSIN (tylvalosin tartrate) Water Soluble Granules in pigs when administered at 50, 150,

and 250 ppm (1, 3, and 5 times the labeled tylvalosin inclusion rate) for 15 consecutive days (3 times the labeled duration of treatment).

b. *Test Animals and Treatment Groups*: Thirty-six weaned healthy pigs (18 non-castrated males and 18 females) were acclimated to the test facility for at least 25 days prior to commencement of treatment. All pigs were Landrace/Large White crosses and were 4 to 8 weeks old on arrival. On arrival, body weights ranged from 6.3 to 13.9 kg.

The animals were individually housed in identically-sized pens with a floor space area of 2 m^2. Temperature and relative humidity were recorded daily.

During the course of the study, all animals were offered an *ad libitum* amount of commercially available, non-medicated feed once daily. Water was offered to all animals via an individual drinking system.

Thirty-two animals (16 non-castrated males and 16 females) were selected for enrollment and were randomly assigned to one of four treatment groups blocked by body weight and gender. The table below outlines the study design (treatment groups):

Table III.1: Margin of Safety Study Design – Treatment Groups

Treatment Groups[1]	Tylvalosin Inclusion Rate in Drinking Water	Number of Animals	Duration of Dosing (Consecutive Days)
1 (0X)	0 ppm	4 Male/ 4 Female	15
2 (1X)	50 ppm	4 Male/ 4 Female	15
3 (3X)	150 ppm	4 Male/ 4 Female	15
5 (5X)	250 ppm	4 Male/ 4 Female	15

[1] Multiples of the labeled tylvalosin inclusion rate in drinking water in parentheses

c. *Test and Control Article Administration*: The test article was AIVLOSIN (tylvalosin tartrate) Water Soluble Granules, formulated as the intended commercial product. Non-medicated tap water was used as the control article. Drinking water, either tylvalosin-medicated or non-medicated tap water was supplied to each animal *ad libitum* via individual drinking water systems for 15 consecutive days (Study Days 1 through 15). Drinking water was replaced with freshly prepared solutions every 24 hours.

Following 15 consecutive days of treatment, all animals received non-medicated tap water for one day.

d. *Measurements and Observations*: The following measurements and observations were made for each animal during the study:

1) Physical examinations, conducted by a masked veterinarian, on Study Days -12, -2, 2, 6, 11, and 15 and included, but were not limited to, evaluation of: general appearance and behavior; integument; musculoskeletal system; cardiovascular system; respiratory system; gastrointestinal system; urinary system; reproductive system;

lymphatic system; nervous system; ocular system; rectal body temperature (°C); heart rate (beats/min); respiration rate (breaths/min); and capillary refill rate (sec).

2) General health observations twice daily (AM and PM) for abnormal behavior or signs of ill health from Day -14 to necropsy.

3) On Study Day 1, additional general health observations at 2, 4, and 6 hours (± 30 minutes) from the time when the first animal received medicated water.

4) Fecal consistency on Study Days -7, -2, 2, 6, 11, and 15.

5) Feed consumption daily from Study Day -7 until necropsy.

6) Water consumption daily from Study Day -14 until necropsy.

7) Body weights on arrival, on Study Days -12, -5, -2, 2, 6, 11, 15, and prior to necropsy.

8) Blood samples for hematology, coagulation, and clinical chemistry examinations on Study Days -12, -2, 2, 6, 11, and 15.

9) Samples for urinalysis and urine microscopy on Study Days -10, -3, 4, 9, and 14.

10) Fecal samples for occult blood analysis within 3 days of arrival and on Study Days -10,-3, 4, 9, and 14.

11) Gross pathology and selected tissue samples for histopathology at necropsy.

4. Statistical Methods: Organ weights were analyzed by analysis of variance with body weight as a covariate and treatment, sex, and treatment by sex interactions as fixed effects. Weights of sex organs were analyzed using a model with body weight and treatment only.

   Repeated measures analysis of variance was used for variables measured at multiple times: body weight, hematology and clinical chemistry variables, and coagulation values. The model included a baseline value as a covariate, treatment, time, and gender and their two and three-way interactions as fixed effects, and animal nested in treatment group as a random effect. The covariance structure was selected based on the lowest Akaike Information Criterion value. Contrasts to compare treatments to control were selected based on the significance of the treatment and treatment interaction effects.

5. Results:

   a. *Tylvalosin Water Concentrations*: All tylvalosin medicated water solutions administered to the pigs during the study assayed within ±10% of the nominal concentration. No tylvalosin was detected in samples of water administered to the control group.

b. *Mortality*: All pigs survived to the scheduled necropsy with the exception of one animal in Treatment Group 2 (1X) which was euthanized on Study Day 8 due to lameness unrelated to treatment.

c. *Body Weights*: There was no statistically significant difference between the body weights of animals from any treated group and the control group.

d. *Feed Consumption*: The average daily feed consumption was statistically significantly higher ($p = 0.007$) in Treatment Group 2 (1X) animals compared to the control group. This finding was considered incidental and not treatment-related. No other statistically significant differences between treated groups and the control group were noted.

e. *Water Consumption*: There was a decrease in water consumption from Day -1 to Day 1 in Treatment Group 3 and Treatment Group 4 animals. Water consumption in both groups returned to normal on Day 2 and continued to be normal throughout the rest of the study. No other statistically significant differences between treated groups and the control group were noted.

f. *Physical Examinations and Clinical Findings*: All animals remained in good general health for the duration of the study, with the exception of one animal euthanized due to non-treatment related lameness (see Mortality above). A small number of sporadic health abnormalities were recorded throughout the course of the study, none of which were of clinical concern or considered to be a result of treatment. Additional health observations at approximately 2, 4, and 6 hours after the initial offering of medicated water resulted in no abnormal observations.

g. *Physical Examination Variables*: There were no clinically relevant changes noted in heart rate, respiratory rate, or capillary refill rate. Four animals had body temperatures of 40.5, 40.3, and 41.3 (Treatment Group 3), and 40.1 °C (Treatment Group 2). These elevations were observed on a single day each, and were attributed to the stress of handling during procedures or a minor inflammatory process and not related to treatment.

h. *Hematology*: Only one hematology parameter (mean cell hemoglobin concentration [MCHC]) had a treatment group by sex interaction that was statistically significant. The adjusted mean MCHC value was significantly lower ($p = 0.053$) in Treatment Group 2 males compared with the control group males. This finding was not considered related to treatment based on lack of dose response (it occurred in only one male pig in Treatment Group 2), the small magnitude of the decrease (MCHC values were 30.7 g/dL on Day 6 and 31.1 g/dL on Day 11 compared to the male study reference range of 31.2 to 33.9 g/dL for MCHC), and the lack of biological significance (no correlative signs of anemia in the study pigs).

i. *Coagulation*: Only one coagulation parameter (prothrombin time [PT]) had a treatment group by time interaction that was statistically significant. The adjusted mean PT value was significantly lower in Treatment Group 2 compared with the control group on Day 2 ($p = 0.063$); however, all PT values in Treatment Group 2 were within the combined study reference

range of 10.0 to 11.8 seconds. On Day 11 and Day 15, the adjusted mean PT value was significantly higher in Treatment Group 3 compared with the control group ($p = 0.067$ and $p = 0.027$, respectively). On Day 11, one male pig in Treatment Group 4 had a PT value of 12.5 seconds, and on Day 15, one male pig in Treatment Group 4 had a PT value of 12.4 seconds. These differences in PT were transient, lacked a clear dose or treatment relationship, and were not accompanied by clinical signs. The changes were not considered to be related to treatment.

j. *Clinical Chemistry*: Statistically significant differences between the control group and one or more treated groups were observed for sodium, chloride, phosphate, urea, triglycerides, lactate dehydrogenase, and albumin. These findings were considered incidental, sporadic, not dose-related, and the individual values from the treated group animals remained within or close to the study reference range. No findings were considered to be related to treatment.

k. *Urinalysis*: The adjusted mean specific gravity was statistically significantly higher in Treatment Group 3 compared with the control group on Study Days 9 and 14 ($p = 0.002$ and $p = <0.001$, respectively). The urinalysis findings were without a clear dose or treatment relationship and were not accompanied by clinical signs, therefore, the changes were not considered to be related to treatment.

l. *Fecal Consistency*: No animal exhibited "watery" feces, and scores of "semi-solid" were only observed in two animals post-treatment (both in Treatment Group 3) on Day 11 and Day 15. The changes in fecal consistency were not considered to be related to treatment.

m. *Fecal Cytology*: Fecal occult blood was confirmed on six observations in five different pigs (one pig in Treatment Group 1, two pigs in Treatment Group 2, and two pigs in Treatment Group 3) post-treatment. These findings were minimal, sporadic, lacked any dose relationship, and were consistent in frequency and clinical presentation with pre-treatment observations of fecal occult blood. These observations were not considered to be related to treatment.

n. *Gross Pathology*: All findings were typical of spontaneously occurring background pathology in pigs of this age. There were no gross necropsy findings related to treatment.

o. *Microscopic Pathology*: All findings were typical of spontaneously occurring background pathology in pigs of this age. There were no histopathology findings related to treatment.

p. *Organ Weights*: There were statistically significant differences between the control group and treated groups in relation to pituitary gland (Treatment Group 4), spleen (Treatment Groups 3 and 4), and heart weights (Treatment Group 3). No conclusion could be drawn for pituitary weights as many were lost or destroyed as a result of the euthanasia procedure; however, there were no clinical signs relating to pituitary dysfunction observed during the study. The differences in spleen and heart weights lacked macroscopic or microscopic correlates, lacked any

clinical signs of heart/splenic disease, and were not considered to be related to treatment.

q. *Organ weights as a Percentage of Brain Weights*: Mean organ weights expressed as a percentage of brain weight were similar across all treatment groups for all organs with the exception of the ovaries. The ovary weight differences are not considered clinically relevant for the currently intended target animal population.

6. Conclusions: This study demonstrated that AIVLOSIN Water Soluble Granules is safe when administered at 50 ppm tylvalosin in the drinking water of swine for 5 consecutive days.

IV. HUMAN FOOD SAFETY:

A. Microbial Food Safety (Antimicrobial Resistance):

Microbial food safety information (antimicrobial resistance) for tylvalosin was evaluated using a qualitative risk assessment procedure. The dosage regimen evaluated was 50 ppm tylvalosin in the drinking water of swine for 5 consecutive days. The indication associated with this dosage regimen is, "For the control of porcine proliferative enteropathy (PPE) associated with *Lawsonia intracellularis* infection in groups of swine in buildings experiencing an outbreak of PPE."

The qualitative risk assessment procedure involved conducting 1) a *release assessment* to describe the probability that tylvalosin and its use in swine will result in the emergence of macrolide-resistant bacteria or macrolide resistance determinants in treated swine under proposed conditions of use; 2) an *exposure assessment* to describe the likelihood of human exposure to macrolide-resistant bacteria or macrolide resistance determinants through consumption of edible products from treated swine; and 3) a *consequence assessment* to describe potential human health consequences arising from exposure to macrolide-resistant bacteria or macrolide resistance determinants by considering the human medical importance of macrolides used in the treatment of human infectious diseases.

It was determined that the risk of development of transferable macrolide resistance elements from this use of tylvalosin in swine is low. This decision is supported by data from animal studies that have shown tylvalosin administration does not cause changes in tylvalosin susceptibility within *Campylobacter* spp. Also, changes have not been demonstrated among *Enterococcus* spp., and, due to pre-existing macrolide resistance, this is of less significance.

Macrolides are ranked as critically important drugs in human medicine; therefore, by default, the consequence assessment yields a high ranking. The overall risk estimation is derived to be high. The conditions of use and restriction of use to only *groups of swine in buildings experiencing an outbreak of PPE* are compatible with the Agency's risk management strategies associated with a product having an overall risk estimation of high.

Decision Statement: The Agency's integration of the degree of risk derived from the three individual assessments (medium, medium, and high) gave an overall risk estimation of high. The conditions of use are compatible with the

Agency's risk management strategies for a Category 1 drug, corresponding to the estimated high risk. Further, post-approval monitoring may be achieved from testing of surrogate antimicrobials (erythromycin and azithromycin) in the current NARMS program.

B. Impact of Residues on Human Intestinal Flora:

The effects of tylvalosin residues on human intestinal flora were assessed through the following approaches.

1. Determination of the need for establishing a microbiological Acceptable Daily Intake (ADI)

a. Step 1: Are residues of tylvalosin and (or) its metabolites microbiologically active against representatives of the human intestinal flora?

Yes, it has been concluded that tylvalosin and its residues are active against representative human intestinal flora. An *in vitro* study was performed to confirm the conclusion and to help calculate the activity. A brief summary of the study is illustrated below.

Study title: Activity of acetylisovaleryltylosin and 3-acetyltylosin against bacterial strains representing the normal human intestinal microbiota: determination of minimum inhibitory concentration (MIC).
Study Number: DWS P1/015/04
Report Date: January 28, 2005
Study Director: Andrew Pridmore, BSc, Ph.D.
Study Location: Don Whitley Scientific Limited, Shipley, West Yorkshire, United Kingdom

Study Summary: Susceptibility testing of tylvalosin and its metabolite 3-acetyltylosin was performed against 100 bacterial isolates (10 isolates from each of 10 genera) representative of human intestinal flora. The isolates came from feces of healthy, non-medicated human volunteers. The methodology used was the agar dilution method as described in Clinical and Laboratory Standards Institute (CLSI) guidelines performed at a single inoculum level as recommended in CLSI guidelines. ATCC strains of *Bacteroides fragilis* (25285), *Eubacterium lentum* (43055), *Staphylococcus aureus* (29213), and *Enterococcus faecalis* (29212) were used to monitor performance and reproducibility of the testing.

Results and Conclusions: The *in vitro* activity of the two compounds against representative bacterial groups is summarized in Table IV.1. Except for *Escherichia coli*, the compounds showed potent and moderate activities against organisms tested. Using a cutoff value of 8 mcg/mL, 9 of the 10 groups were included in the calculation of overall activities for each compound based on MIC_{50}s. The mean MIC_{50} values for the susceptible genera were 0.335 mcg/mL for tylvalosin and 0.256 mcg/mL for 3-acetyltylosin.

b. Step 2: Do tylvalosin residues enter the human colon?

Yes, by default, it is concluded that tylvalosin and its metabolites enter the human colon.

Table IV.1. Summary of susceptibility of tylvalosin and its metabolite 3-acetyltylosin

Bacterial Group	Tylvalosin MICs (mcg/mL)			3-acetyltylosin MICs (mcg/mL)		
	MIC_{50}	**MIC_{90}**	**MIC Range**	**MIC_{50}**	**MIC_{90}**	**MIC Range**
Bacteroides fragilis	0.5	2	0.25-2.0	1.0	8	0.5-8
Bacteroides spp.	0.5	4	0.125-4	1.0	4	0.125-32
Bifidobacterium spp.	0.06	0.125	0.06-0.125	0.06	0.5	0.06-0.5
Clostridium spp.	0.25	0.5	0.06-0.5	0.06	0.25	0.06-0.5
Eubacterium spp.	0.5	0.5	0.5-1.0	0.25	0.5	0.25-2
Fusobacterium spp.	4	8	0.5-32	0.5	2	0.125-32
Peptostrepto-coccus	0.5	0.5	0.125-1.0	0.5	1.0	0.25-4
Enterococcus spp.	1.0	8	1-8	2	8	1-8
E. coli	>128	>128	>128	>128	>128	>128
Lactobacillus spp.	2	>128	0.5->128	4	>128	0.5->128
All isolates	**0.5**	**>128**	**0.06->128**	**0.5**	**>128**	**0.06->128**

c. Step 3: Do tylvalosin residues entering the human colon remain microbiologically active?

Yes, with support from study data, a fraction of tylvalosin residues entering the human colon remains biologically active. The study is summarized below.

Study title: Effect of fecal binding on antibacterial activity of acetylisovaleryltylosin and 3-acetyltylosin.
Study Number: DWS P1/016/04
Report Date: February 23, 2005
Study Director: Andrew Pridmore, BSc, Ph.D.
Study Location: Don Whitley Scientific Limited, Shipley, West Yorkshire, United Kingdom

Study Summary: The objective of the study was to determine the effect of fecal binding on the antibacterial activity of tylvalosin and 3-acetyltylosin. Stock solutions of tylvalosin and 3-acetyltylosin were prepared freshly on the day of experiment. Selected concentrations of both compounds (0, 1, 2, 5, 10, 20, 50, and 100 mcg/mL) prepared in microbiological culture medium were incubated with increasing concentrations of sterilized human feces (0, 10, 25, and 50% w/v) from 3 separate healthy male and female donors. The mixtures were incubated for 30 minutes, 1, 2, 6, or 8 hours. Fecal solids from each mixture were removed by centrifugation at each time period. The supernatants of the mixtures from each test compound/fecal slurry were inoculated with *Enterococcus faecalis* (ATCC 29212) and incubated for 24 hours to assess antibacterial activity of the compound in the supernatant. Growth of the test strain was estimated by turbidity or presence of a cell pellet in inoculated cells compared to non-inoculated control cells of each fecal/drug mixture. This comparison gave an indication of the unbound drug or metabolite in each preparation.

Results and Conclusions: Concentrations of fecal slurries from 10% to 50% showed apparent effects of solids on binding, and maximum binding was seen with 50% feces. Without exposure to feces, each test compound inhibited *E. faecalis* growth at a concentration of 1 mcg/mL. With 50% feces, approximately 90% of binding occurred soon after incubation began and maximum binding (at least 98%) occurred between 0.5 hours (mostly for 3-acetyltylosin) and 6 hours of interaction (tylvalosin) for 2/3 donors (the third donor had 98% binding at 0 incubation time). Thus, it was concluded that up to 2% of the fraction of tylvalosin residues in the fecal environment remain biologically active.

d. Step 4: Determination if there is any scientific justification to eliminate testing for either one or both endpoints of concern:

- **Colonization barrier disruption**

  It was concluded that a microbiological ADI (mADI) is to be determined based on this endpoint.

- **Changes in resistant populations**

  Enterococcus spp. were used as index intestinal bacteria for assessing whether there is scientific justification to eliminate this endpoint. A mADI for resistance is not needed for tylvalosin residues because macrolides are not used in the treatment of enterococcal infections, and decreased activity for macrolides already exists in the enterococcal population from humans.

2. Determination of the final Microbiological ADI

a. Determination of the fraction of oral dose available to microorganisms

From the fecal binding study described in Step 3 (above), it was determined that the fraction of the oral dose of tylvalosin residues available to microorganisms is 2%.

b. Determination of the Microbiological ADI using MIC_{calc}

The mADI was determined based on the following formula with MIC_{calc}.

$$ADI\,(mcg/kg\,BW/day) = \frac{MIC_{calc}\ X\ Mass\ of\ colon\ content}{Fraction\ of\ dose\ available\ X\ 60\,kg}$$

MIC_{calc}s of tylvalosin and 3-acetyltylosin derived from the MIC study (Study # DWS P1/015/04) were 0.335 mcg/mL and 0.256 mcg/mL, respectively. The smaller of the two was used as the overall MIC_{calc} for tylvalosin. Using the formula, the following mADI of tylvalosin is determined:

mADI = (0.26 x 220)/(0.02 x 60) = 47.67 mcg/kg BW/day

Therefore, the mADI for tylvalosin is **47.7 mcg/kg BW/day, or 2.86 mg/person/day**.

C. Toxicology:

1. Summary of Toxicology Studies

Toxicity tests determining the human food safety of tylvalosin are summarized below:

a. Type of Study: Thirteen-Week In-Feed Toxicity Study in Mice

Report Number: 16939
Report Date: July 8, 1999
Study Director: T. Martin, BSc, MSc, MIBiol, CBiol
Study Location: Inveresk Research, Tranent, Scotland

Experimental Design and Conclusion: This study was conducted to evaluate the potential systemic toxicity of tylvalosin when administered daily in the diet to groups of ten male and ten female CD-1 mice for 90 days at dose levels of 0, 207, 2075, 20750 ppm in feed. Body weights and food consumption were measured weekly; animal observations were conducted twice daily; and hematology and biochemical determinations were performed during week thirteen of treatment. At the termination of the study, mice were sacrificed and full macroscopic post mortem examinations were performed. Organs were weighed, preserved, and subjected to microscopic evaluation.

No treatment related effects on mortality, food consumption, or eye toxicity were reported. Clinical signs such as piloerection, hunched posture, and hyperactive behavior were seen in the high-dose group and in the intermediate dose females. The high-dose group had decreased body weight on the first week and a decrease in food consumption. There was also a slight decrease in mean body weight gain in the low dose males and intermediate dose males and females with no decrease in food consumption. Slight decreases in several organ weights (heart, liver, kidney, and pituitary gland, etc.) were seen in intermediate and high-dose animals but without histopathological findings. Increases in total bilirubin, decreases in glucose levels, total protein, hematocrit, and hemoglobin concentration occurred in the high-dose males. A decrease in hematocrit was also seen in the intermediate group. The No Observed Effect Level (NOEL) for this study was 207 ppm, equivalent to 38.5 mg/kg BW/day for males and 45.5 mg/kg BW/day for females, based on the clinical signs (hunched posture, hyperactive behavior), decreased body weight gain, and decreased hematocrit in females in the next higher dose level tested of 2075 ppm.

b. Type of Study: 13-Week Toxicity (feeding) Study in Rats

Study Number: 86-102
Report Date: October 30, 1987
Study Director: Yoshihiko Ito
Study Location: Research Institute for Animal Science in Biochemistry and Toxicology, Shimo-kuzawa, Sagamihara-shi, Kanagawa-ken

Experimental Design and Conclusion: Tylvalosin was administered daily in the diet to groups of ten male and ten female Crj: CD (SD) SPF rats for 90 days at concentration of 0, 329, 1645, 8225, and 41125 ppm in feed. Clinical signs, food consumption, body weights, ophthalmoscopic examinations, hematology, clinical biochemistry, urine analysis, organ weights, and histopathological examination were obtained.

At 41125 ppm, food intake decreased immediately after the treatment and gradually recovered after a couple of weeks. Weight gain was significantly below the control throughout the study. Blood chemistry parameters and hematology parameters also were affected. Necropsy of the surviving animals showed distention of the cecum; discoloration of the adrenal gland; and increased weight of the thyroid, pituitary, and salivary glands. The thyroid gland had vacuolation of the follicular epithelium and the adrenal gland showed vacuolation in the zona glomerulosa. At 8225 ppm, food intake was slightly reduced only on week one of treatment. Body weight gain was also slightly reduced throughout the study in females. In addition, a decrease in serum total bilirubin, an increase in thyroid and pituitary absolute and relative weights, and vacuolation of the follicular epithelium of the thyroid gland occurred in males of the 8225 ppm group. Three males and six females died in the 8225 and 41125 ppm groups during treatment. No treatment-related effects were reported for the lower dose groups. A NOEL of 1645 ppm, equivalent to 111.8 mg/kg BW/day for males and 130.9 mg/kg BW/day for females, was determined based on a decrease in serum total bilirubin, increases in thyroid and

pituitary absolute and relative weights, and vacuolation of the follicular epithelium of thyroid gland at the next higher dose of 8225 ppm in feed.

c. Type of Study: 13-Week Toxicity Study in Dogs

Study Number: TOX.UK.050051
Report Date: August 16, 2006
Study Director: Leo Ayerakwa
Study Location: Huntingdon Life Sciences Ltd., Alconbury, Huntingdon, Cambridgeshire, England

Experimental Design and Conclusion: Tylvalosin was administered to groups of four male and four female beagle dogs by oral capsule for 90 days at dose levels of 0, 40, 80, and 160 mg/kg BW/day. All dogs were dosed one hour before feeding. Clinical signs, food consumption, body weights, ophthalmoscopic examinations, hematology, clinical biochemistry, urine analysis, organ weights, and histopathological examination were obtained. Loose and liquid feces were observed at the mid and high-dose levels. Tylvalosin also caused vomiting at the mid and high-dose levels and salivation at the high-dose level. Liquid feces and vomiting were more pronounced in treated females. A NOEL of 40 mg/kg BW/day was determined based on treatment-related increases in incidence of liquid feces and vomiting seen at the next higher level of 80 mg/kg BW/day.

d. Type of Study: 52-Week Oral Toxicity Study in Beagle Dogs

Study Number: 1272-004 (TOX.US.070138)
Report Date: June 8, 2009
Study Director: Duane W. Poage, M.S.
Study Location: MPI Research, Inc., Mattawan, Michigan

Experimental Design and Conclusion: This study was conducted to evaluate the effects of tylvalosin following daily oral capsule administration to beagle dogs for one year. Four male and four female beagle dogs were administered vehicle or tylvalosin at 20, 80, or 160 mg/kg BW/day for 52 consecutive weeks. Morbidity/mortality, cageside observations, detailed clinical observations, and ophthalmoscopic examinations were conducted. Body weights, food and water consumption, and body temperature were measured. Electrocardiographic (ECG) examinations were conducted. Fasted blood samples and urine samples for clinical pathology evaluations were collected from all animals. Blood samples were also collected for determination of the toxicokinetic parameters. At treatment termination, all surviving animals were euthanized with sodium pentobarbital and full necropsy examinations were performed. Organs were weighed, preserved, and subjected to microscopic evaluation.

Treatment-related effects associated with the mid (80 mg/kg BW/day) and high-dose levels (160 mg/kg BW/day) were clinical observations (salivation and watery feces). There were no treatment-related effects associated with the low-dose level (20 mg/kg BW/day), which was determined to be the NOEL.

e. Type of Study: Oral (Gavage) Development Toxicity Study in Mice

Study Number: Tabulated Study Report No. 18
Report Number: 16768
Report Date: May 26, 1999
Study Director: S. C. Clubb
Study Location: Inveresk Research, Tranent, Scotland

Experiment Design and Conclusion: In order to detect effects on embryonic and fetal development in pregnant CD-1 mice, tylvalosin was administered by oral gavage, once daily, from day 6 through to day 20 post coitum at dose levels of 0, 166, 332 or 581 mg/kg BW/day. Significant maternal toxicity was observed in the high-dose group with death and clinical observations including but not limited to piloerection, excessive salivation, hunched appearance, irregular respiration, and subdued behavior. Similar clinical effects were also observed in the mid-dose group of 332 mg/kg BW/day. Because no effects were observed at the low-dose group, the maternal developmental toxicity NOEL of 166 mg/kg BW/day was determined.

Eye opening at fetal stage and altered rates of ossification (e.g., skull bone, hyoid bone, 5th metacarpal and metatarsal, cervical vertebrae, and sternebrae) associated with the treatment of 332 and 581 mg/kg BW/day dose groups were observed. Additional effects included increased incidence of displaced testis in the 581 mg/kg BW/day group and a dose-related decline in number of fetuses with thirteen complete ribs. A fetal developmental toxicity NOEL of 166 mg/kg BW/day was determined based on the altered ossification and observed eye opening in fetuses at the next higher dose level of 332 mg/kg BW/day.

f. Type of Study: Rat Two-Generation Reproduction Toxicology Study

Study Number: Tabulated Report # 20
Report Number: 17053
Report Date: September 7, 1999
Study Director: S. J. Barton
Study Location: Inveresk Research, Tranent, Scotland

Experiment Design and Conclusion: A two-generation reproduction study was conducted at dose levels of 18, 90, and 450 mg/kg BW/day in the diet.

Weight gain was reduced in the high-dose level of 450 mg/kg BW/day in the F_0 and F_1 generation and also in the mid-dose level of 90 mg/kg BW/day in F_0 females. The high-dose level and the mid-dose level caused reduced numbers of implantation sites in F_0 females. The high-dose level caused reduced litter weights and individual pup weights in both generations while the mid-dose level caused reduced litter weight and individual pup weight in the F_1 generation males. Treatment at the lowest dose level of 18 mg/kg BW/day did not have significant effects on reproduction in rats. Therefore, a NOEL for reproductive effects was established at 18 mg/kg BW/day based on reduced numbers of

implantation sites, litter weights, and individual pup weights at the next higher dose level of 90 mg/kg BW/day.

Although histopathology was not performed for several tissues in the F_0 in this study, including thyroid, the study was considered adequate for evaluation of the reproduction toxicity of tylvalosin because adequate histopathology was performed on tissues and organs from the 13-week rat study at similar doses.

g. Type of Study: Bacterial Reverse Mutation Assay using *Salmonella typhimurium/Escherichia coli* (Ames Test)

Report Number: #21
Study Investigator: Akihiro Yoshimoto
Study Location: Central Research Laboratory, Sanraku Co, Ltd., Japan
Study Completion Date: December 12, 1987

Experimental Design and Conclusion: The study was conducted to determine the genotoxicity of tylvalosin. Three test methods i) standard plate incorporation method, ii) centrifugal method, and iii) DNA recombination test using bacteria were applied to assess the mutagenic potential of tylvalosin. i) Plate incorporation method was based on the direct plate method of Ames *et al.*, (Mutation Research 31, 347-364. 1975). Tester strains employed were *S. typhimurium* TA98, TA100, TA1535, and TA1537, and *Escherichia coli* strain WP2 *uvr*A. The assay was conducted both with and without a phenobarbital and 5, 6-benzofuran-induced rat (SD rats) liver S9. It is noted that no records for the estimation of protein content of the S9 as well as results on any other biological/biochemical properties of the S9 used in the assay were maintained. Test doses ranged from 0.781 mcg/plate to 800 mcg/plate. ii) The bacterial test organisms were collected by centrifuge in the centrifugal method. Doses employed in the test ranged from 0.25 mcg/plate to 5000 mcg/plate. iii) DNA recombination test using bacteria is based on the method described by Kada *et al.* (Chemical Mutagens. Vol 6. Plenum, New York, 1980). Bacterial strains used in the test were *Bacillus subtilis* M45 Rec^- and H17 Rec^+. Doses employed in the test ranged from 0.20 mcg to 100 mcg. There was no difference in the anti-bacterial activity of tylvalosin against both strains.

The test item neither precipitated nor showed signs of bacteriotoxicity. Under the testing conditions used and applying standard mutagenicity criteria, tylvalosindid not show evidence of a mutagenic potential.

h. Type of Study: Bacterial Reverse Mutation Assay using *Salmonella typhimurium/Escherichia coli* (Ames Test)

Report Number: AB07CY.503.BTL
Study Investigator: V. Wagner and M. VanDyke
Study Location: BioReliance, Rockville, Maryland
Study Completion Date: November 28, 2005

Experimental Design and Conclusion: The study was conducted to determine the genotoxicity of tylvalosin.

Direct plate method of Ames *et al.*, (Mutation Research 31, 347-364. 1975) was applied in the study using four strains of *S. typhimurium*, i.e., TA 1535, TA 1537, TA 98, TA100, and one strain of *E. coli*, i.e., WP2*uvr*A (pKM101). The metabolic activation system (S9 mix) consisted of liver homogenate (S9) from Aroclor-1254 induced male Sprague-Dawley rat liver and the necessary cofactors. Toxicity of the test substance was assessed in a preliminary toxicity-mutation assay with test doses ranging from 1.5 mcg/plate to 5000 mcg/plate in the presence and absence of S9 mix. Precipitate was observed at 5000 mcg/plate. The test article was toxic to the bacteria from 15 mcg/plate to 5000 mcg/plate. Due to excessive toxicity, tester strains TA1535 and TA1537 were retested in the absence of S9. Based on this test, the top dose used in the confirmatory mutation assay was 1500 mcg/plate for tester strains TA 98, TA100, and WP2*uvr*A (pKM101) in the presence of S9 and 150 mcg/plate with all strains in the absence of S9 mix. With TA1535 and TA1537, dose levels used in the presence of S9 ranged from 0.15 to 150 mcg/plate. No precipitate was observed but toxicity was observed at 15, 50, 150, or 500 mcg/plate. Under the conditions of this study, tylvalosin did not cause a positive response in the presence or absence of Aroclor-induced rat liver S9.

i. Type of Study: *In Vitro* Mammalian Cell Gene Mutation Test (L5178Y/TK$^{+/-}$ Mouse Lymphoma Assay)

Study Number: AB07CY.704.BTL (BioReliance)
Study Investigator: Jane J. Clarke
Study Location: BioReliance, Rockville, Maryland
Report Date: December 02, 2005

Experimental Design and Conclusion: The study was conducted to determine the mutagenic potential of tylvalosin.

Mouse lymphoma L5178Y cells, clone 3.7.2C, were used in the assay. Physiological saline was used as the solvent for the test article. A preliminary toxicity test was performed to establish the optimal dose levels for the mutagenesis assessment. In the non-activation phase of the mutation assay using 4 hours of exposure, concentrations ranging from 5 to 100 mcg/mL were tested; in the initial non-activation phase of the mutation assay using 24 hours of exposure, concentrations ranging from 5 to 50 mcg/mL were tested; in the confirmatory non-activation phase of the mutation assay using 24 hours of exposure, concentrations ranging from 10 to 60 mcg/mL were tested; in the initial S9-activation system using 4 hours of exposure, concentrations from 50 to 250 mcg/mL were tested; in the confirmatory S9-activation system using 4 hours of exposure, concentrations from 150 to 250 mcg/mL were tested. The induction of forward mutation at the thymidine kinase (TK) locus in the presence and absence of S9 was assayed by colony growth of L5178Y/TK mouse lymphoma cells.

In the initial 4-hour exposure assay with S9, 150 and 200 mcg/mL doses exhibited an average mutant frequency (MF) of 121 and 118, respectively, compared to average MF of 47.5 in the vehicle control. One culture treated with 150 mcg/mL and one culture treated with 200 mcg/mL

exhibited an induced mutant frequency of ≥90 mutants per 10^6 clonable cells over that of the solvent control. In the confirmatory assay with S9, one cloned culture treated with 200 mcg/mL of test article exhibited an induced mutant frequency of ≥90 mutants per 10^6 clonable cells over that of the solvent control. Similarly, in the confirmatory 24-hour exposure assay, -S9, the average MF at 50 and 60 mcg/mL doses was 100 and 138, respectively, compared to the average MF in vehicle control of 63.5. The mutagenic response was stronger in the presence of S9 compared to the response seen in the absence of S9.

Tylvalosin produced a weak positive response in the initial and confirmatory 4-hour exposure (+S9) assay and in the confirmatory 24-hour exposure (-S9). The report did not contain colony size information on those doses that exhibited weak mutagenic responses in the assay needed to determine whether the response observed was due to gene mutations or due to chromosome damage.

j. Type of Study: A Micronucleus Study in Beagle Dogs

Report Number: 1272-005
Study Investigator: Scott Boley
Study Location: One year toxicology study was conducted at MPI Research, Inc. Michigan and the micronucleus analysis was performed at Litron Laboratories, Rochester, New York
Report Date: November 4, 2008

Experimental Design and Conclusion: *In vivo* micronucleus study in beagle dogs (Study Number: 1272-005) was conducted in conjunction with a one year chronic toxicity study (Study Number: 1272-004). Micronucleus measurements were made in the peripheral blood from beagle dogs exposed with doses of 20, 80, and 160 mg/kg BW. Peripheral blood was drawn from the treated animals 72 hours after the treatment and analyzed for micronucleated reticulocytes (MN-RETs) by flow cytometry (Harper *et al.*: Toxicological Sciences 100(2), 406-414. 2007). The positive control group was administered a single dose of cyclophosphamide intravenously at a concentration of 1.25 mg/kg BW, and peripheral blood from these animals was assayed for MN-RETs 48 hours later. There were no significant differences in dogs treated with blank capsules or tylvalosin. Tylvalosin did not induce micronuclei in the peripheral blood of treated beagle dogs under the current experimental conditions.

k. Type of Study: *In Vitro* Mammalian Chromosome Aberrations Test using Chinese Hamster Ovary (CHO) Cells

Project Number: 761485
Author: E. Murie
Study Location: Inveresk Research International, Scotland
Report Date: October 30, 1998

Experimental Design and Conclusion: The study was conducted to determine the potential of tylvalosin to induce chromosomal aberrations.

Clone CHO-10-B4 of the CHO Cells were exposed to tylvalosin, vehicle control, or positive control for 6 hours in the presence of S9 mix and for 22 hours in the absence of S9 mix in two experiments. Cultures were harvested at 24 hours in the first experiment and 48 hours post treatment in the second experiment. In the first experiment, nine dose levels ranging from 20 to 5000 mcg/mL were tested with the presence of S9. Tylvalosin was toxic to the cells at 156 mcg/mL and above. Without S9, the test article was toxic to the cultures at 78 mcg/mL and above. A positive response was noted in the number of aberrant cells excluding gaps in culture treated with 313 mcg/mL. In the absence of S9, all tylvalosin treated cultures gave negative responses. Based on the results of the first experiment, the following dose levels were selected for the second experiment: +S9: 39, 78, 117, 156, 234, 313, 469, and 625 mcg/mL; and -S9: 20, 39, 78, 117, 156, 234, and 313 mcg/mL.

In the second experiment, cultures treated with doses from 234 mcg/mL to 625 mcg/mL showed signs of toxicity with the presence of S9. Cultures treated with 469 mcg/mL and 625 mcg/mL were too toxic for metaphase analysis. Positive responses were noted for all parameters in both cultures treated with 234 mcg/mL and 313 mcg/mL. There was a dose related increase in the numbers of aberrant cells.

In the absence of S9, toxicity was noted in cultures treated with doses from 78 mcg/mL to 313 mcg/mL. Cultures treated with 234 mcg/mL and 313 mcg/mL were too toxic for metaphase analysis. In one culture in 117 mcg/mL dose and one culture in 156 mcg/mL dose, positive responses were noted in the number of lesions per cell and the number of aberrant cells excluding gaps.

Because positive responses were observed in the 24 hour harvest cultures, cultures from 48 hour harvest were not conducted. It is concluded that tylvalosin was clastogenic under the test conditions used in the chromosomal aberration test with the CHO cells.

l. Type of Study: *In Vivo* Bone Marrow Mouse Micronucleus Assay in CD-1 Mice

Project Number: IRI 762190
Study Investigator: L. M. Holmstrom and D.C. Innes
Study Location: Inveresk Research International, Scotland
Report Date: October 23, 1998

Experimental Design and Conclusion: The study was conducted to determine the *in vivo* mutagenic potential of tylvalosin in the bone marrow of CD-1 mice. Dose range finding and main toxicity tests were conducted prior to the micronucleus study.

In the dose range test, mice (one animal/sex/group) were dosed orally at 0 hour and 24 hours with concentrations of 50, 125, 350, 800, and 2000 mg/kg BW. Based on the clinical signs of toxicity observed from the dose range study (subdued behavior, hunched appearance, piloerection, and partially closed eyes etc), exposure levels of 800, 1200, and 1600 mg/kg BW were chosen for the main toxicity study of 3

animals/sex/group. Test article induced mortality was noted at 1200 and 1600 mg/kg BW. Accompanying clinical signs were piloerection, subdued behavior, labored breathing, prostration, convulsions, tremors, hunched appearance, and partially closed eyes, etc. From the results of the main toxicity study, doses of 200, 400, and 800 mg/kg BW were selected for the micronucleus test.

In the main micronucleus study, five male CD-1 mice were treated with the doses of 200 or 400 mg/kg BW and ten male and ten female mice were treated with 800 mg/kg BW. Six animal deaths occurred in the high-dose group. Clinical signs of subdued behavior, agitation, rolling gait, soiled coat, staggering, hunched appearance, and partially closed eyes, etc. were observed following treatment. The numbers of micronucleated bone marrow polychromatic erythrocytes (MNPCE) in mice dosed with the vehicle control were not significantly different with the numbers of MNPCE recorded for the treatment groups. There was no bone marrow toxicity in any of the treatment groups. It is concluded that tylvalosin did not induce micronuclei in bone marrow cells when tested to the maximum tolerated dose of 800 mg/kg BW/day in male and female CD-1 mice under the testing conditions used.

m. Type of Study: *In Vivo* Bone Marrow Cytogenetics Assay in Mice

Project Number: AB07CY.108.BTL
Study Investigator: Ramadevi Gudi and Ljubica Krsmanovic
Study Location: BioReliance, Rockville, Maryland
Report Date: December 30, 2005

Experimental Design and Conclusion: The study was conducted to determine the *in vivo* clastogenic potential of tylvalosin.

ICR mice (5 animals/sex/group) were dosed orally with tylvalosin at 200, 400, and 800 mg/kg BW in a single oral gavage. Cyclophosphamide monohydrate (CP) was used as a positive control. Test article induced mortality was noted at 800 mg/kg BW. Accompanying clinical signs were lethargy, ataxia, excessive salivation, piloerection, convulsions, and grooming.

The mitotic index of bone marrow cells was reduced by 23.8% relative to the vehicle control group in the male group treated with 800 mg/kg BW. The mitotic index was normal compared to the vehicle control group in all other test article-treated groups. No statistically significant increase in the percentage of aberrant cells was observed in the test article-treated groups relative to the vehicle control group.

Under the conditions of this study, tylvalosin did not induce a significant increase in the number of cells with structural or numerical chromosome aberrations. Tylvalosin produced a negative response in the acute *in vivo* cytogenetic assay using male and female ICR mice.

n. Type of Study: *In Silico* Prediction of Potential Toxicological Properties (Computational Toxicology Letter Report)

Authors: Jane Cotterill and Qasim Chaudhary
Study Location: Central Science Laboratory of Defra at Sand Hutton, York, United Kingdom
Report Date: December 2005

Experimental Design and Conclusion: This is a computational toxicology report on the test article tylvalosin.

Tylvalosin was evaluated using DEREK for Windows version 8.0.1 (Lhasa Ltd). DEREK predicts the toxicity end points for species that include, among others, humans. The toxicity end points include, among others, carcinogenicity, and genotoxicity that includes gene mutations and clastogenicity.

The analysis by DEREK on the presence of the structural alerts in the molecule indicated that:

- It is plausible (i.e., there is weight of evidence from *in vitro* studies of compounds with similar structure) that tylvalosin may exhibit mutagenicity and genotoxicity in various species, including humans, due to the presence of an alkyl aldehyde group (or precursor).

- It is considered plausible (i.e., there is weight of evidence from *in vitro* studies of compounds with similar structure) that tylvalosin may cause chromosome damage in various species, including humans, due to the presence of a substituted vinyl ketone group.

- The predictions obtained using DEREK did not indicate any structural alert associated with carcinogenicity.

Based on the Structure Activity Relationship (SAR) analysis, it was predicted that tylvalosin exhibits structural alerts for genotoxic response, but not carcinogenicity.

Although positive responses were observed in some genetic toxicology studies, it was concluded that tylvalosin is not likely to be carcinogenic to humans based on a weight-of-evidence approach that considered all available toxicology information, with particular emphasis on the results of all genetic toxicity studies and the one-year chronic dog study.

2. Determination of Toxicological No Observed Effect Level (NOEL) for chronic exposure

The NOEL for tylvalosin in toxicity studies was 18 mg/kg BW/day based on toxicity endpoints from a two-generation reproductive oral toxicity study in rats.

3. Determination of Toxicological ADI

Applying a safety factor of 100 to this NOEL derived from a chronic study, an ADI is calculated as shown below.

$$ADI = \frac{NOEL}{Safety\ Factor} = \frac{18\ mg/kg\ bw/day}{100} = 0.18\ mg/kg\ bw/day = 180\ mcg/kg\ bw/day$$

D. Assignment of the Final ADI:

Because the microbiological ADI (47.7 mcg/kg BW/day) is lower than the toxicological ADI (180 mcg/kg BW/day), the microbiological ADI is the final ADI for total AIVLOSIN residues.

E. Safe Concentrations for Total Residues:

30% of the ADI (14.3 mcg/kg BW/day) was allocated to edible tissues. A safe concentration in muscle is calculated from the acceptable daily intake, assuming the average weight of a man to be 60 kg and the daily human intake of muscle to be 300 g, as follows:

$$SC = \frac{ADI \times HumanWeight}{FoodFactor} = \frac{14.3\ mcg/kg\ bw/day \times 60\ kg}{300\ g/day} = 2.9 mcg/g = 2.9\ ppm$$

The safe concentrations for total residues in liver, kidney, and fat are calculated using food factors of 100 g for liver, and 50 g for kidney and fat as follows:

Liver: 8.6 ppm

Kidney: 17.3 ppm

Fat: 17.3 ppm

F. Residue Chemistry:

1. Summary of Residue Chemistry Studies

a. Total Residue and Metabolism Studies

(1). Study Number and Title

Inveresk Report Number 16922: Metabolism and residue depletion of [^{14}C]-tylvalosin in piglets.

(2). Study Dates

May 1998 to March 1999

(3). Study Director and Location of Study

GC Speirs, BSc, PhD; Inveresk Research, Tranent, Scotland

(4). Identity of Substance and Dosage Form

[^{14}C]-tylvalosin in capsules

(5). Test Animals

Sixteen Landrace piglets, weighing 9.7 – 13.4 kg at dosing commencement, were divided into four groups, with two males and two females in each group.

(6). Levels and Duration of Dosing

[^{14}C]-tylvalosin was administered as a single daily dose for 7 consecutive days at 2.5 mg tylvalosin/kg BW.

(7). Route of Administration

Oral, via gelatin capsule

(8). Study Design

Two male and 2 female pigs were sacrificed at 12 hours and 1, 3, and 5 days after the last dose. Edible tissues and plasma were analyzed. In addition, the pigs sacrificed at 5 days after the last dose were held in separate metabolism cages. Urine and feces samples were collected throughout the study at 24 hour intervals from this group and analyzed.

(9). Samples Analysis

Total radioactivity was determined using liquid scintillation counting and combustion. Metabolite profiling was conducted using a validated HPLC method.

(10). Results

The major route of elimination for pigs was via feces. Maximum concentrations of total radioactivity were observed in most tissues at 12 hours after the last dose. The concentrations of total radioactivity in the edible tissues are shown in Table IV.2.

Table IV.2. Mean Total Radioactivity in Tissues Following Oral Administration of [^{14}C]-Tylvalosin Once Daily for 7 Days at a Dose Rate of 2.5 mg/kg Body Weight

Tissue	Total Residue Concentration (mcg equiv./g)							
	12 Hours		1 Day		3 Days		5 Days	
	Males	Females	Males	Females	Males	Females	Males	Females
Fat*	0.172	0.203	0.081	0.048	0.046	0.039	0.031°	0.033°
Kidney	0.335	0.280	0.190	0.215	0.136	0.079	0.096	0.090
Liver	0.295	0.670	0.214	0.254	0.112	0.068	0.065	0.116
Muscle	0.020	0.038	0.010°	0.013°	0.005°	0.004°	0.005°	0.005°
Skin**	0.075	0.145	0.125	0.114	0.031°	0.064°	0.058	0.070
Plasma	0.017	0.026	0.010	0.007	0.011°	0.004	0.008°	0.004°

*: Fat Composite
**: Skin with Fat
°: Mean includes individual results less than 30 disintegrations per minute above background.

The nature of radioactivity in the liver and kidney samples of one male and one female animal at 12 hours and of one female animal at 1 day after the last dose was investigated. Information regarding components in these samples with chromatographic properties similar to those of tylvalosin and 3-acetyltylosin (3-AT), a metabolite of tylvalosin, is summarized in Table IV.3. Up to 8 other components were also found in the liver and kidney samples, each of which accounted for approximately 1-32% of the total residues in the samples. However, these components could not be identified.

Table IV.3. Quantities of Tylvalosin and 3-AT in Liver and Kidney at 12 hours and 1 day Post Last Dose Following 7 Consecutive Daily Oral Administrations of [^{14}C]-Tylvalosin to Piglets

Sample Type	3-AT (% TRR)	AIV (% TRR)
(#1) Male Liver 12 h	5.8	8.8
(#4) Female Liver 12 h	8.1	6.3
(#7) Female Liver 1 day	5.4	4.7
(#1) Male Kidney 12 h	9.6	6.6
(#4) Female Kidney 12 h	8.9	16.0
(#7) Female Kidney 1 day	13.3	5.5

b. Comparative Metabolism Study

(1). Metabolism Study in Rats

(a). Study Number and Title

Inveresk Report Number 16673: Metabolism and distribution of [^{14}C]-tylvalosin in rats.

(b). Study Dates

May 1998 to August 1998

(c). Study Director and Location of Study

NW McCracken, BSc, PhD, Inveresk Research, Tranent, Scotland

(d). Identity of Substance and Dosage Form

[^{14}C]-Tylvalosin in sterile water

(e). Test Animals

Fourteen Sprague Dawley rats, 6-7 weeks old and weighing 216 to 293 g at dosing commencement.

(f). Levels and Duration of Dosing

[^{14}C]-Tylvalosin was administered as a single daily dose for 7 consecutive days at 20 mg tylvalosin/kg BW.

(g). Route of Administration

Oral, via gastric gavage

(h). Study Design

Table IV.4. Study Design for Investigating Total Residues and Metabolism of [^{14}C]-Tylvalosin in Rats

Treatment	Phase	No. of rats	Blood/excreta samples	Tissue samples
[^{14}C]-tylvalosin 20 mg/kg BW by oral gavage for 7 days	1	3M+3F	Plasma 0.25, 0.5, 1, 2, 4 and 6 h post-dose Day 1 and pre-dose Days 2-7	3 h after last dose
[^{14}C]-tylvalosin 20 mg/kg BW by oral gavage for 7 days	2	3M+3F	Urine and feces, for 24 h periods throughout treatment period and for 5 days thereafter	144 h after last dose
[^{14}C]-tylvalosin 20 mg/kg BW by oral gavage for 7 days	2	1M+1F	Urine and feces as above, plus expired air for 24 h periods, on Days 1 and 2 of treatment	144 h after last dose

(i). Sample Analysis

Total radioactivity was determined using liquid scintillation counting and combustion. Metabolite profiling was conducting using a validated HPLC-MS procedure.

(j). Results

Plasma concentrations of total radioactivity were at or near the limit of detection at each measurement time, suggesting only limited systemic absorption. There was no increase in plasma total radioactivity with multiple dosing, suggesting that accumulation of radiolabeled components did not occur on repeated dosing.

The majority of the dose (>85%) was recovered in feces. Radioactivity excreted in urine accounted for 1-5% of the total administered dose in male and female rats. There were no gender related differences seen in tissue concentrations of total radioactivity. HPLC analysis of urine and feces pools from male and female rats indicated that tylvalosin was extensively metabolized; up to ten components were detected in these samples. However, of these components, only tylvalosin and metabolite 3-AT were identified.

(2). Metabolism Study in Piglets

See results reported in "a. Total Residue and Metabolism Studies"

(3). Comparison of Tylvalosin Metabolites in Piglets and Rats

The sponsor submitted information on comparison of tylvalosin metabolite profiles in piglets and rats based on estimated retention times of HPLC peaks for components of the residues found in piglets and rats. CVM did not require unequivocal identification of the unknown residue components other than tylvalosin and 3-AT because of the low total residue concentrations found in edible tissues of pigs at zero withdrawal relative to the safe concentrations.

c. Study to Establish Withdrawal Period

(1). Study Number and Title

Report Number 010-01143: Determination of Tylvalosin Residues in Liver of Swine Receiving AIVLOSIN Water Soluble Granules (62.5% w/w Tylvalosin) for 5 Consecutive Days in Drinking Water Containing 50 ppm Tylvalosin

(2). Study Dates

July 2010 to August 2010

(3). Study Director and Location of Study

In-life Phase: JW Byrd, Southwest Bio-Labs, Inc., Las Cruces, NM

Analytical (tissues): XenoBiotic Laboratories, Inc, Plainsboro, NJ

Analytical (water): Lancaster Laboratories, Inc, Lancaster, PA

(4). Test Material and Dosage Form

Tylvalosin in the commercial formulation - AIVLOSIN Water Soluble Granules (62.5% w/w Tylvalosin) administered in drinking water.

(5). Test Animals

Ten York cross-bred pigs (5 barrows and 5 gilts), approximately 6 months old, weighing 277 to 298 lbs at 1 day prior to dosing, were used in the study. The animals were divided into a control group (one barrow and one gilt) and a treated group (4 barrows and 4 gilts).

(6). Dose and Route of Administration

For 5 consecutive days, animals were given *ad libitum* access to medicated drinking water containing, on average, 49.2 ppm tylvalosin. Fresh medicated water was prepared daily.

(7). Tissue Sample Collection and Analysis

Animals were slaughtered within 3 hours after the last treatment. Tylvalosin concentrations in liver were determined for each animal using a validated HLPC-MS-MS procedure.

(8). Results

The reported parent tylvalosin residue concentrations in swine liver were in the range of 0.65 ng/g to 31.5 ng/g, all of which were below the Limit of Quantitation (LOQ) of the method (50 ng/g).

(9). Conclusions

The study results are consistent with assigning a zero-day withdrawal for the use of AIVLOSIN (tylvalosin tartrate) Water Soluble Granules when administered at 50 ppm tylvalosin in the drinking water of swine for 5 consecutive days.

2. Target Tissue and Marker Residue

It is not necessary to assign a target tissue or a marker residue for tylvalosin residues in swine.

3. Tolerance

A tolerance for tylvalosin in swine is not required.

4. Withdrawal Period

No withdrawal period is required (i.e., zero withdrawal).

G. Analytical Method for Residues:

A regulatory analytical method for monitoring tylvalosin residues in swine is not required.

V. USER SAFETY:

The product labeling contains the following information regarding safety to humans handling, administering, or exposed to AIVLOSIN Water Soluble Granules:

WARNINGS:
NOT FOR HUMAN USE.
KEEP OUT OF REACH OF CHILDREN.

USER SAFETY WARNINGS:

May cause skin irritation.

Tylvalosin Tartrate has been shown to cause hypersensitivity reactions in laboratory animals. People with known hypersensitivity to Tylvalosin Tartrate should avoid contact with this product. In case of accidental ingestion, seek medical advice.

When handling AIVLOSIN Water Soluble Granules and preparing medicated drinking water, avoid direct contact with the eyes and skin. Wear a dust mask, coveralls and impervious gloves when mixing and handling this product. Eye protection is recommended. In case of accidental eye exposure, wash eyes immediately with water. If irritation persists, seek medical attention. Avoid eating, chewing gum and smoking during handling.

Wash contaminated skin.

The Material Safety Data Sheet contains more detailed occupational safety information. To report adverse effects in users, to obtain more information or obtain a Material Safety Data Sheet, call the ASPCA Animal Product Safety Service at 1-800-345-4735.

VI. AGENCY CONCLUSIONS:

The data submitted in support of this NADA satisfy the requirements of section 512 of the Federal Food, Drug, and Cosmetic Act and 21 CFR part 514. The data demonstrate that AIVLOSIN Water Soluble Granules, when used according to the label, is safe and effective for the control of porcine proliferative enteropathy (PPE) associated with *Lawsonia intracellularis* infection in groups of swine in buildings experiencing an outbreak of PPE. Additionally, data demonstrate that residues in food products derived from swine treated with AIVLOSIN Water Soluble Granules will not represent a public health concern when the product is used according to the label.

A. Marketing Status:

Labeling restricts this drug to use by or on order of a licensed veterinarian (Rx marketing status). Adequate directions for lay use cannot be written because (a) professional expertise is required to appropriately diagnose and subsequently use this product to treat PPE and (b) restricting this drug to use by or on the order of a licensed veterinarian should help prevent indiscriminate use which could result in violative tissue residues.

B. Exclusivity:

Under section 512(c)(2)(F)(i) of the Federal Food, Drug, and Cosmetic Act, this approval qualifies for FIVE years of marketing exclusivity beginning on the date of the approval because no active ingredient of the new animal drug has previously been approved.

C. Patent Information:

For current information on patents, see the Animal Drugs @ FDA database or the Green Book on the FDA CVM internet website.